Cytogenetic and Molecular Characterization of Random Chromosomal Rearrangements Activating the Drug Resistance Gene, *MDR1/P-Glycoprotein,* in Drug-Selected Cell Lines and Patients With Drug Refractory ALL

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Drug resistance, both primary and acquired, is a major obstacle to advances in cancer chemotherapy. In vitro, multidrug resistance can be mediated by P-glycoprotein (PGY1), a cell surface phosphoglycoprotein that acts to efflux natural products from cells. PGY1 is encoded by the *MDR1* gene located at 7q21.1. Overexpression of *MDR1* has been demonstrated in many cancers, both in patient tumors and in cell lines selected with a variety of chemotherapeutic agents. Recent studies in drug-selected cell lines and patients samples have identified hybrid mRNAs comprised of an active, but apparently random, gene fused 5′ to *MDR1*. This observation indicates that random chromosomal rearrangements, such as translocations and inversions, leading to "capture" of *MDR1* by constitutively expressed genes may be a mechanism for activation of this gene following drug exposure. In this study, fluorescence in situ hybridization (FISH) using whole chromosome paints (WCP) and bacterial artificial chromosome (BAC)-derived probes showed structural rearrangements involving 7q in metaphase and interphase cells, and comparative genomic hybridization (CGH) revealed high levels of amplification at chromosomal breakpoints. In an adriamycin-selected resistant colon cancer line (S48–3s/Adr), WCP4/WCP7 revealed t(4;7)(q31;q21) and BAC-derived probes demonstrated that the breakpoint lay between *MDR1* and sequences 500–1000 KB telomeric to it. Similarly, in a subline isolated following exposure to actinomycin D (S48–3s/ActD), a hybrid *MDR1* gene composed of heme oxygenase-2 sequences (at 16p13) fused to *MDR1* was identified and a rearrangement confirmed with WCP7 and a subtelomeric 16p probe. Likewise, in a paclitaxel-selected MCF-7 subline where CASP sequences (at 7q22) were shown to be fused to *MDR1*, WCP7 showed an elongated chromosome 7 with a homogeneously staining regions (hsr); BAC-derived probes demonstrated that the hsr was composed of highly amplified *MDR1* and CASP sequences. In all three selected cell lines, CGH demonstrated amplification at breakpoints involving *MDR1* (at 7q21) and genes fused to *MDR1* at 4q31, 7q22, and 16p13.3. Finally, in samples obtained from two patients with drug refractory ALL, BAC-derived probes applied to archived marrow cells demonstrated that a breakpoint occurred between *MDR1* and sequences 500–1000 KB telomeric to *MDR1*, consistent with a random chromosomal rearrangement. These results support the proposal that random chromosomal rearrangement leading to capture and activation of *MDR1* is a mechanism of acquired drug resistance. *Genes Chromosomes Cancer 23:44–54, 1998.*

INTRODUCTION

The development of resistance to chemotherapeutic agents is a serious problem in the clinical treatment of cancer, particularly since tolerance to one agent is often accompanied by cross-resistance to a variety of other, often unrelated, compounds (Biedler and Riehm, 1970; Bech-Hansen et al., 1976; Pastan and Gottesman, 1987; van der Bliek and Borst, 1989). There is considerable evidence that acquired resistance to multiple natural products in vitro is mediated primarily by P-glycoprotein (PGY1), a cell surface glycoprotein originally described by Ling, that is encoded by the *MDR1* gene (Juliano and Ling, 1976). P-glycoprotein functions as an energy-dependent drug efflux pump that acts to reduce intracellular concentrations of drugs (Beck et al., 1979; Fojo et al., 1985; Krishan et al., 1985). Although increased expression of *MDR1*/P-glycoprotein has been demonstrated in numerous in vitro models and in patient samples, the mechanisms whereby this increase in expression occurs have not been fully elucidated. It should be noted that the evidence to date indicates that

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activation of *MDR1* as a mechanism for resistance is relevant only in cells with very low or undetectable levels of *MDR1*; for cells that express higher levels of *MDR1* de novo, such as those in the adrenal, kidney, and liver, this has not been identified as a mechanism of acquired resistance.

The *MDR1* gene is located at chromosome band 7q21.1 (Fojo et al., 1986; Callen et al., 1987) and previous studies (Slovak et al., 1987; Nieuwint et al., 1992) have reported abnormalities of the long arm of chromosome 7 in drug-resistant cells containing *MDR1* gene amplification and overexpressing PGY1. In studies of hybrid cells resulting from somatic cell fusions between a drug-sensitive human leukemia cell line (CEM/CCRF) and a drug-resistant derived cell line [CEM/A7, selected with doxorubicin (Zalcberg et al., 1994)], only the two hybrids with the chromosomal abnormality involving 7q21 [dup(7)(q11.23q31.2)] showed evidence of PGY1 overexpression (de Silva et al., 1996). Interestingly, these two hybrids did not demonstrate amplification of *MDR1* DNA.

Earlier molecular studies of three drug-resistant cells and bone marrow cells from two acute lymphoblastic leukemia (ALL) patients refractory to therapy showed the formation of hybrid mRNAs comprised of an active, but random, gene fused to the 5' region of *MDR1* (Mickley et al., 1997). These data suggest that cells with constitutional low or undetectable levels of PGY1, when exposed to drug, acquire rearrangements between *MDR1* and variable gene partners that confer on them a selective advantage in a drug-containing environment. This observation suggests that random chromosomal rearrangements involving 7q may be responsible for overexpression of *MDR1* when cells that express low levels of *MDR1* are exposed to drug. In the present study, using fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH), we provide evidence for such a mechanism. We also show that at higher concentrations, in addition to the *MDR1* gene rearrangement, *MDR1* gene amplification occurs. This amplification is demonstrated by CGH, Southern blot, and direct visualization of the two chromosome partners by FISH. In one of the cell lines, FISH with whole chromosome paint (WCP) probes revealed entire chromosomes consisting of many alternating segments of two chromosomes, resulting in a "harlequin" appearance. In another cell line, FISH with bacterial artificial chromosome (BAC)-derived probes showed high levels of amplification of the two genes involved in the rearrangement.

The aim of this study is to provide insights into the chromosomal and molecular changes involving the *MDR1* gene during acquisition of drug resistance, and to characterize the nature of acquired mutations in *MDR1* that lead to overexpression and amplification.

MATERIALS AND METHODS

Tissue Culture

The human colon adenocarcinoma cell line (S48–3s) and its selected sublines (Adr, ActD) were grown in a 1:1 mixture of IMEM + Ham's Nutrient Mixture F12. The Adr 0.1, 1.0, and 10.0 sublines were maintained in 0.1, 1.0, and 10.0 μg/ml adriamycin, respectively. The subline, ActD 0.1, was subcultured in 0.1 μg/ml actinomycin-D. MCF-7 cells were grown in IMEM media. The resistant cell line, MCF-7 TX400, was maintained in medium containing 400 ng/ml paclitaxel.

Chromosomal Mapping Using Somatic Cell Hybrids

Chromosomal assignment of the novel sequence isolated from S48–3s/Adr10.0 and the leukemia sample, ALL *2*, were determined using the PCR and Poly A and Poly D somatic cell hybrids (Polymeropoulos et al., 1993). The 5′ and 3′ primer pairs used for somatic cell hybrids were, for S48-Adr10, 5′-AGCTCGCTCAGCCGCCG-3′ and 5′-TTCCACCGCCTCCTCCA-3′, respectively, and for ALL *2*, 5′-CAGCTTCCACAGGAGCAA-3′and 5′-CGGTTATTTGCTCTAGTTTC-3′, respectively.

Fluorescent In Situ Hybridization

Chromosome preparations of the BrdU-synchronized cell lines were prepared according to previously described cytogenetic techniques (Dutrillaux and Viegas-Pequignot, 1981). FISH was performed using WCP kits for chromosomes 4 and 7 (Vysis, Downers Grove, IL), an alpha satellite probe for chromosome 16 (Vysis), and a subtelomere probe for 16p (National Institutes of Health and Institute of Molecular Medicine Collaboration, 1996). All hybridizations were done overnight and all slides counterstained with 4′,6-diamidino-2-phenylindole (DAPI). The preparations were observed with a Leitz epifluorescence microscope equipped with a Chroma triple-bandpass filter (Texas Red, FITC, and DAPI) and photographed with a Leitz Orthomat E camera and Ektachrome 400 film, or analyzed on a Leica DMRXA microscope equipped with Leica QFISH image analysis system.

FISH analysis using BAC-derived clones (Shizuya et al., 1992) was performed on S48–3s parent and S48–3s/Adr cell lines, MCF-7 parent and MCF-7 TX400 cell lines, and on bone marrow cells from two patients with ALL. For the S48 and ALL cells, the two BAC-derived probes selected were one containing *MDR1* sequences (BAC clone 332G20, Research Genetics, Huntsville, AL) and a probe containing sequences 500–1,000 KB telomeric to *MDR1* (BAC clone 063L14, Research Genetics). These sequences were chosen because we were looking for rearrangements proximal to the 5′ region of *MDR1*, and it is known that *MDR1* is transcribed in a centromeric direction. The ALL cells, which had been stored in DMSO at −70°, were thawed, washed twice with EBBS solution, cultured in RPMI with 10% fetal bovine serum for 4–24 hr, and harvested according to standard cytogenetic techniques. For the MCF-7 lines, the two BAC-derived probes were the same BAC containing *MDR1* sequences as was used for the other sample, and a BAC-derived clone containing sequences from the promoter region of the CASP gene (BAC clone 313A17, Research Genetics), which is located >15 KB telomeric to *MDR1*. The hybridization procedure is similar to other FISH techniques. Briefly, the metaphase slides were pretreated with RNase, pepsin, formaldehyde, and an ethanol series, then denatured at 80°C for 1.5 min, dehydrated in an ethanol series, and air-dried (Ried et al., 1992). Nick-translated probe DNA was labeled with FITC, biotin, or digoxigenin, denatured at 80°C for 3 min, and preannealed to human Cot1 DNA (GIBCO-BRL) at 37°C for 1 hr. After a 2–3 day hybridization at 37°C, detection was carried out in a four-layer antibody procedure, each incubated for 30 min: (1) mouse anti-FITC (DAKO, Denmark) or mouse antidigoxin (Sigma, St. Louis, MO) + avidin TRITC (Vector Laboratories, Burlingame, CA); (2) rabbit anti-mouse FITC (Sigma) + biotinylated goat antiavidin (Vector); (3) avidin TRITC; and (4) goat antirabbit FITC (Sigma). The slides were counterstained with DAPI. Analysis was conducted with a Leica DMRXA microscope equipped with Leica QFISH computer software.

Comparative Genomic Hybridization

CGH was performed according to a modification of the method described by du Manoir et al. (1993). Control DNA was obtained from the lymphocytes of a cytogenetically normal male and the test DNA was extracted from the parent and drug-resistant tumor cell lines. Each sample was nick-translated with digoxigenin or biotin, and DNase was added to produce fragments with a final size of about 500 bp. For each test, 200 ng of digoxigenin-labeled normal (control) DNA and 200 ng of biotin-labeled tissue culture DNA were ethanol-precipitated in the presence of blocking DNA (Cot-1 fraction of human DNA from GIBCO-BRL, Bethesda, MD), which reduces nonspecific hybridization of the probe to nontarget DNA, and carrier DNA (salmon sperm DNA), to reduce background. The control and test DNAs were cohybridized at 37°C to normal metaphase chromosomes for 4 days, followed by detection with fluorescein conjugated to avidin (Vector Laboratories) and antidigoxigenin conjugated to rhodamine (Boehringer Mannheim, Indianapolis, IN). The chromosomes were counterstained with DAPI. Three fluorescent images were acquired from each metaphase—using single-bandpass filters TR1, TR2, and TR3 (Chromo Technology, Brattleboro, VT) for DAPI, FITC, and TRITC, respectively—with a Leica DMRBE epifluorescence microscope. Ratio images and profiles were determined using a custom computer program (du Manoir et al., 1995). A total of 15–20 images were acquired per case, of which five to eight images were used to produce the final CGH profile. For each cell line, the results of the parent line were compared to those obtained from the drug-resistant line.

Reverse Transcription Followed by PCR

One μg of RNA was reverse-transcribed and amplified for 35 cycles according to previously described conditions (Mickley et al., 1997). The *MDR1* and non-*MDR1* primers utilized for RT-PCR are summarized in Table 1.

RESULTS

Molecular Evidence of Gene Rearrangement

This study describes the characterization of three multidrug-resistant cell lines (S48–3s/Adr, S48–3s/ActD, and MCF-7 TX400) and two samples obtained from patients with relapsed and drug refractory ALL. Molecular studies in these cell lines and patient samples were reported in a previous publication (Mickley et al., 1997). All three cell lines and both ALL samples demonstrated chromosomal rearrangements involving *MDR1*, and the cell lines also had amplification of *MDR1* and the respective genes fused to it. These results are summarized in Table 2.

TABLE 1. Primers Used for PCR

Cell line	Gene	5′ primer	3′ primer	PCR product, bp
S48-3s Adr	Chr 4	AGCTCGCTCAGCCGCCG	GCCATCAAAGAAGAAGAACTTC	248
S48-3s ActD	*HO2*	GCGGAAGTGGAAACCTCAGA	AAGTACAAAGGGGCAAAGGC	288
MCF-7 TX400	CASP	ATCAGCCGCTCACTCCGT	CTGCAGCTGCTGTAAATCAA	97
ALL 1	*NRF1*	ATGTGGCTACTTACACCGAG	CCAGGTCTTCCAGGATCATG	419
ALL 2	Chr 1	CAGCTTCCACAGGAGCAA	CGGTTATTTGCTCTAGTTTC	177
All cell lines	*MDR1*	GCCTGGCAGCTGGAAGACAAATACACAAAATT	CAGACAGCAGCTGACAGTCCAAGAACAGGACT	286

TABLE 2. Drug Resistance and Molecular and Cytogenetic Results

Cell lines/ patients	Chromosomes	Genes	FISH (WCP and BAC)	CGH[a]
S48Adr	7q21	*MDR1*	t(4;7) translocation	Amp. 7q21
	4q31–33	unknown		Amp. 4q31–33
S48ActD	7q21	*MDR1*	Translocation t(7;16)	Amp. 7q21
	16p13.3	*HMOX2*[b]		Amp. 16p13
MCF-7 TX400	7q21	*MDR1*	Amp. of *MDR1* and CASP in long hsr in chromosome 7	Amp. 7q21
	7q22	CASP of *CDP*[c]		Amp. 7q22
ALL 1	7q21	*MDR1*	Rearrangement (BAC probes)	Not done
	7q31	*NRF1*		
ALL 2	7q21	*MDR1*	Rearrangement (BAC probes)	Not done
	chromosome 1	unknown		

[a]Amp., amplification.
[b]*HMOX2,* heme oxygenase-2.
[c]*CDP,* CAAT displacement protein; CASP, *CDP* alternately spliced product.

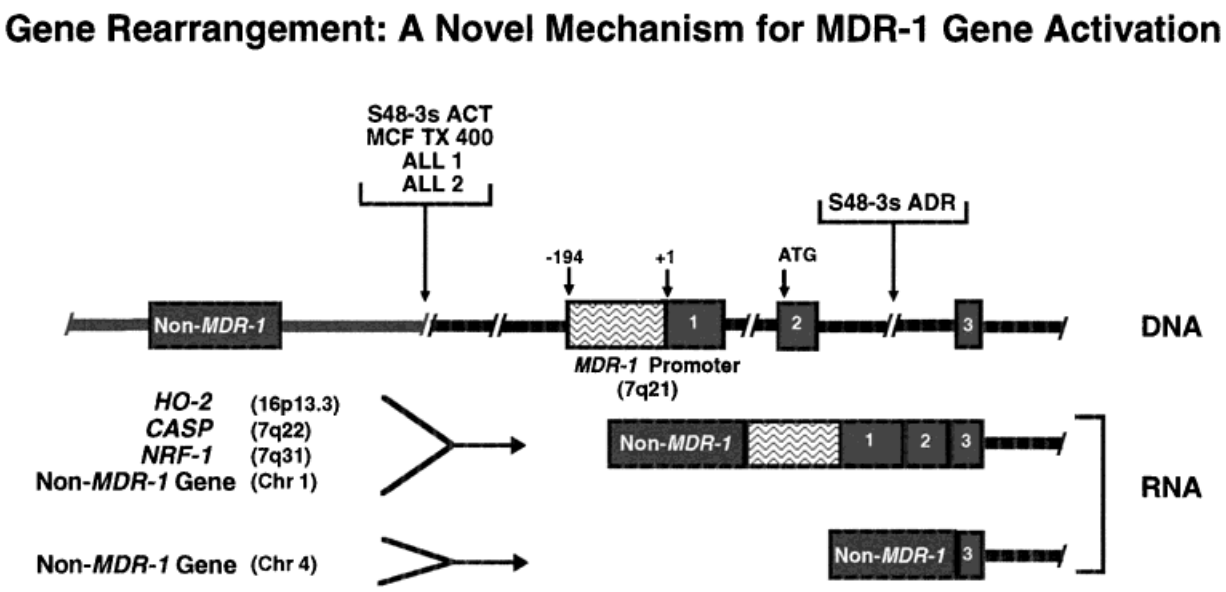

Figure 1. Summary of molecular data in tissue culture cell lines and patient samples: schematic of known or putative sites of rearrangements.

The initial investigation had identified a rearrangement in an adriamycin-selected human colon carcinoma cell line, designated S48–3s/Adr. Overexpression of *MDR1* was demonstrated to occur concurrent with the observed molecular rearrangement, and similar rearrangements were found both in additional cell lines and in patient samples. Figure 1 summarizes the molecular data, which is presented as a schematic of the known or putative sites of rearrangement. The rearrangement identi-

fied in the S48–3s/Adr cell line was shown to be in intron 2 of *MDR1*, and resulted in disruption of the normal gene sequence (deletion of the first 21 amino acids), but produced a functional protein. All the other *MDR1* rearrangements occurred proximal to the normal start of transcription, and resulted in a hybrid *MDR1* mRNA, but a normal protein. The non-*MDR1* sequences fused to *MDR1* were isolated using the 5' RACE methodology. This allowed the identification of the sequences upstream of the normal start site of transcription (or in the case of S48–3s cells, proximal to residue 202, which was identified as the most 5' *MDR1* residue). A search of existing databases for the sequences 5′ of *MDR1* identified some of them as belonging to known genes localized to specific chromosomal locations: CASP (*CDP* alternately spliced product), 7q22 (Scherer et al., 1993); *HO2* (heme oxygenase-2), 16p13.3 (Kutty et al., 1994); and *NRF1* (nuclear respiratory factor-1), 7q31 (Gopalakrishnan and Scarpulla, 1995). The cloned sequences not found in the database were localized to specific chromosomes using somatic cell hybrids (not shown). For S48–3s/Adr cells, somatic cell hybrid analysis localized the non-*MDR1* sequence to chromosome 4, while for one of the two acute leukemia samples, chromosome 1 was identified as the normal site of the non-*MDR1* gene.

The results of the FISH analysis, using WCPs and BAC-derived probes, and the CGH data are presented in Figures 2–7. In each of the cell lines, the CGH profiles of the parent and drug-resistant clones were very similar, with the exception of *MDR1* and the regions determined to be amplified as a result of chromosomal rearrangement involving the *MDR1* gene. Our results with the parent MCF-7 breast cancer cell line were similar to the MCF-7 profile obtained by Kallioniemi et al. (1994). Specifically, copy number increases were seen in 5p, 8q21–qter, 14q, 17q22–qter, and 20q13.

S48–3s/Adr

In this adriamycin-selected cell line, overexpression of *MDR1* was the result of a translocation between chromosomes 4 and 7, t(4;7)(q31;q21); the gene on chromosome 4 is unknown, while the 7q21 breakpoint corresponds to the location of *MDR1*. Figure 2A demonstrates FISH using WCP4 and WCP7 in the parental cells, which have two copies of chromosome 4 and three copies of chromosome 7. Figure 2B–F shows the results in the series of S48–3s cell lines, which were derived from parental S48–3s cells by stepwise selection in increasing concentrations of adriamycin. In the first step of the selection, designated S48–3s/Adr0.1, a balanced reciprocal translocation is seen involving the long arms of chromosomes 4 and 7 (Fig. 2B). Amplification of the hybrid gene occurred and increased with advancing selection, producing alternate chromosome 4/chromosome 7 painting: low levels of amplification are seen in the cells maintained in 1 μg/ml adriamycin (S48–3s/Adr1.0) (Fig. 2C and D), while in the highly resistant cell line maintained in 10 μg/ml adriamycin (S48–3s/Adr10) one or more chromosomes with multiple alternating sequences from 4 and 7 were observed. In some cases, entire chromosomes consisting of many alternating segments of both chromosomes were seen, resulting in a "harlequin" or bright, multicolored appearance (Fig. 2E and F).

BAC hybridization was performed as described in the Materials and Methods section using a BAC containing *MDR1* sequences and a BAC with sequences 500–1,000 KB telomeric to *MDR1*. Hybridization in the S48–3s parent line showed merged signals on both chromosomes 7 (Fig. 2G). Hybridization in the S48–3s/Adr10 line showed clear evidence of a break telomeric to *MDR1* as a result of the 4;7 translocation. Fig. 2H shows merged signals (no rearrangement) on two normal chromosomes 7, while the third set of BAC signals are split from one another with the *MDR1* containing BAC (red) remaining on chromosome 7, and the BAC telomeric to *MDR1* translocated to chromosome 4 (green). It should be noted that only one *MDR1* allele is amplified, the other allele remains as a single copy. CGH on the resistant cell line, S48–3s/Adr10 (Fig. 3), shows amplification at 4q31 and 7q21, consistent with the location of the chromosomal breakpoint revealed by the FISH analysis. The concomitant amplification of 4q31 and 7q21 is consistent with the juxtaposition of these two chromosomal segments by translocation followed by amplification of this two-chromosome hybrid segment.

S48–3s/ActD

In this actinomycin-D-selected S48–3s cell line, the sequences 5′ of *MDR1* were identified as belonging to the heme oxygenase-2 gene, localized to 16p13.3. Attempts to demonstrate a rearrangement involving chromosome 7 and chromosome 16 failed when a whole chromosome 16 paint was used, probably because of the small size of the material translocated from chromosome 16. However, rearrangements with juxtaposition of telomeric sequences from chromosome 16 to chromosome 7 were successfully demonstrated with a 16p subtelomeric probe. The parental cells (S48–3s) (Fig. 4A) have three chromosomes 7 (WCP-7), and two normal copies of chromosome 16 with two subtelomeric 16p sites. In the resistant subline

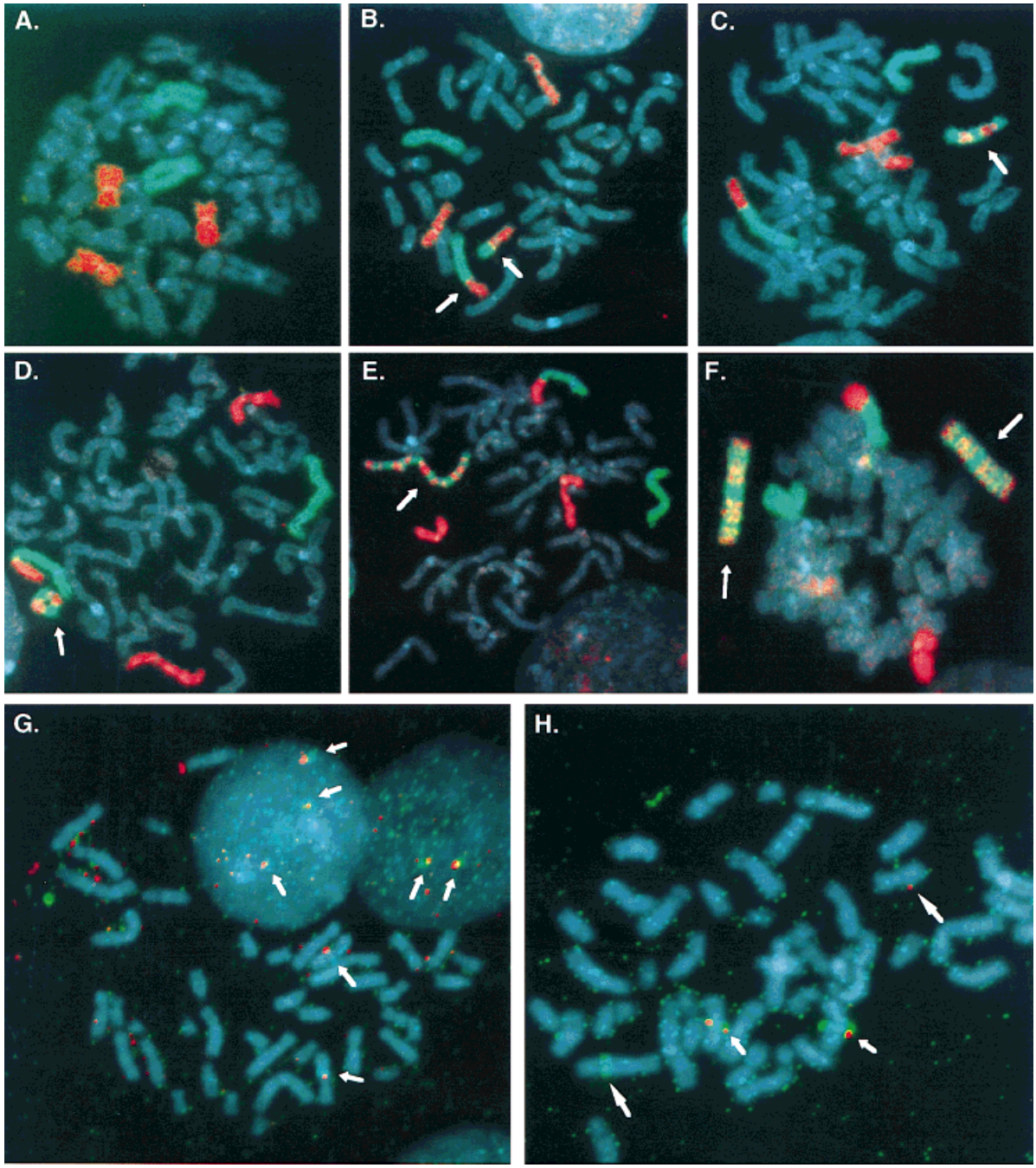

Figure 2. S48–3s and S48–3s/Adr. **A:** Whole chromosome paint (WCP) 4 (green) and 7 (red) in S48–3s parent. **B:** WCP4 and WCP7 in S48–3s/Adr 0.1: one chromosome 4, two chromosomes 7, and reciprocal t(4;7) (arrows). **C** and **D:** WCP4 and WCP7 in S48–3s/Adr 1.0: one chromosome 4, two chromosomes 7, t(4;7), and one chromosome showing alternating segments of 4 and 7. In D, the alternating segments are in the form of a ring chromosome. **E** and **F:** WCP4 and WCP7 in S48–3s/Adr 10: nonrearranged copies of 4 and 7, and t(4;7). In E, one chromosome shows many alternating segments of 4 and 7 (arrow), whereas in F, two chromosomes show ''harlequin'' appearance of alternating 4 and 7 segments (arrows). **G** and **H:** FISH with BAC-derived probes in S48–3s parent line and S48–3s/Adr10 clone: the BAC containing *MDR1* sequences is shown in red; the second BAC-derived probe telomeric to *MDR1* is shown in green. Merged signal = no rearrangement; split signal = disjunction of BAC-derived probes secondary to a rearrangement. For G, S48–3s parent: only merged signals are seen in metaphase and interphase cells (arrows); for H, S48–3s/Adr10: merged signals on two normal chromosomes 7 (arrows); t(4;7)(q31;q21) is indicated by green signal alone on der(4) (arrow) and red signal alone on der(7) (arrow).

(S48–3s/ActD) (Fig. 4B), juxtaposition of these sequences occurs, with several translocations between chromosome 7 and 16p being observed with advancing drug selection, consistent with low levels of amplification in this cell line; one of the chromosomes 16 has lost the 16p subtelomeric sequences. The CGH profile (Fig. 5) demonstrates low levels of amplification of both 7q and the 16p13 region.

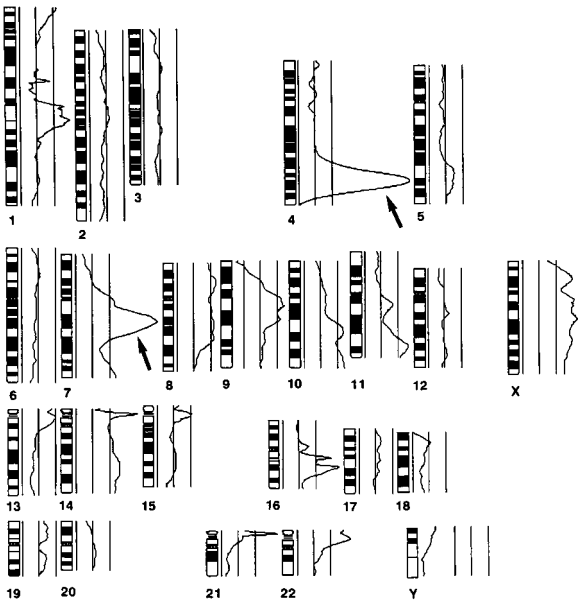

Figure 3. CGH in S48–3s/Adr10. Note amplification at 4q31 and 7q21 (arrows).

MCF-7 TX400

This resistant cell line was derived from parental MCF-7 cells by selection with paclitaxel (Taxol®). The sequences 5' to *MDR1* belong to CASP, an alternately spliced variant of the *CDP* (CAAT displacement protein) gene, located at 7q22. Since this gene is on chromosome 7, FISH analysis using WPC7 did not demonstrate a rearrangement. However, as shown in Figure 6, compared to parental MCF-7 cells (Fig. 6A), the resistant cells (Fig. 6B) have a long chromosome 7 consistent with a homogeneously staining region comprised of sequences from chromosome 7, and BAC-derived probes for the two genes (*MDR1* and CASP) demonstrated that the hsr is comprised of sequences from both the *MDR1* gene and CASP (Fig. 6C). Although FISH analysis demonstrated amplification of chromosome 7 sequences, it could not resolve whether the sequences from *MDR1* and CASP were coamplified. CGH analysis (Fig. 6D and E) revealed coamplification of both the 7q21 (*MDR1*) and 7q22 (CASP) regions of chromosome 7 with what is most likely reduced or absent amplification of the intervening regions. Absence of amplification of the intervening regions would be consistent with juxtaposition of CASP sequences close to *MDR1* as a result of deletion of the intervening sequences or a chromosomal inversion.

Acute Lymphoblastic Leukemia Samples

Bone marrow cells from two patients with relapsed ALL had been harvested and stored after the patients failed treatment regimens, which included natural products known to be P-glycoprotein substrates (daunomycin, vincristine, and etoposide). Molecular cloning studies in these two samples had previously identified sequences from the *NRF1* gene (7q31) (ALL patient 1) and from chromosome 1 (ALL patient 2) proximal to *MDR1*. Since there was a high likelihood that adequate chromosomal spreads would not be obtained from the archived frozen leukemia cells to allow metaphase FISH analysis to be performed, we utilized a sensitive FISH assay for interphase nuclei, using two BAC-derived probes (one probe containing *MDR1* sequences and a second probe containing sequences 500–1,000 KB telomeric to *MDR1*) that would permit the identification of rearrangements telomeric to *MDR1*. These two probes were chosen because previous studies had described transcription of *MDR1* as proceeding in a centromeric direction, and also because of the expectation that rearrangements resulting in capture of *MDR1* by an active gene are likely to occur 5′ to the start site of transcription (as found in S48–3s/ActD and MCF-7 TX400). Both samples (Fig. 7A and B) demonstrated one normal chromosome 7 with colocalization of both signals plus evidence of a rearrangement as shown by signal disjunction in all or a majority of the cells; these results indicate that rearrangements had occurred 5′ of *MDR1*, leading to displacement of chromosomal sequences recognized by the telomeric clone to a different chromosome or elsewhere on chromosome 7, separating the two signals. Interestingly, one sample (patient 2) had been previously identified as expressing very high levels of *MDR1* in about 70% of blasts by in situ hybridization, and in this sample the disruption of signal colocalization was detected in 70% of the blasts examined.

DISCUSSION

In this study we present cytogenetic evidence supporting chromosomal rearrangement as a mechanism for the development of multidrug resistance. We also demonstrate that FISH using WCP or BAC-derived probes may be a useful tool for detecting and predicting acquired drug resistance in clinical settings. The gene rearrangements described involved the multidrug-resistant gene *MDR1*. Transcription of *MDR1* was enhanced when it became fused distal to apparently random genes. Studies of numerous cell lines and normal tissues demonstrated that the genes to which *MDR1* was fused were constitutively expressed in all RNA samples examined (data not shown). Thus, the rearrangements led to increased *MDR1* expression

and, in the cell lines, this was followed by amplification as the cells were exposed to increasing concentrations of chemotherapeutic agents. Each of the three cell lines (S48–3s/Adr, S48–3s/ActD, and MCF-7 TX400) was selected with a different chemotherapeutic agent (adriamycin, actinomycin, and paclitaxel), while the two ALL patients had received a variety of drugs known to be PGY1 substrates. The partner gene was different in each of the five cases.

The past few years have seen intensive efforts devoted to understanding and overcoming the problem of clinical drug resistance. Multidrug resistance mediated by P-glycoprotein, a cell surface phosphoglycoprotein encoded by the *MDR1* gene, has been extensively studied and characterized in vitro and identified in many refractory tumors. While increased expression has been identified in drug-resistant cells, the mechanisms whereby this increased expression occurs have not been fully elucidated. In the course of examining different P-glycoproteins for acquired mutations, we detected a deletion in the *MDR1* gene of one cell line (S48–3s/Adr) secondary to a chromosomal translocation and proposed that it represented a novel mechanism of acquired drug resistance (Mickley et al., 1995). Further studies identified similar rearrangements in additional cell lines and patient samples, and established gene rearrangement as a novel mechanism of *MDR1* gene activation (Mickley et al., 1997).

The identification of chromosomal rearrangements as responsible for activation of gene expression is not a novel finding, e.g., in Burkitt's lymphoma (Taub et al., 1982), but has not been previously reported to involve a gene encoding a drug resistance protein. In a majority of chromosomal rearrangements in cancer, activation involves specific partners, but examples of "promiscuous" chromosome loci rearranging with specific chromosome breakpoints (such as 3q27, 8q24, and 22q11) are well known in cancer cytogenetics. In this study of rearrangements involving *MDR1*, the results indicate that the gene juxtaposed to *MDR1*, which controls its expression following the rearrangement, is chosen randomly. The only requirement for the partner gene appears to be that it be constitutively active. However, it is possible that these genes may possess particular characteristics that are currently unrecognized, such as the existence of stress response elements in their promoter regions. In three examples (CASP, *HO2*, and *NRF1*), the hybrid mRNAs start near the normal start of transcription, supporting the concept that the hybrid gene is under the transcriptional control of the non-*MDR1* gene (Mickley et al., 1995). One can envision that, depending on the non-*MDR1* gene's promoter elements, different inducibilities and transcriptional controls may be observed. This may provide an explanation for why inducibility of *MDR1* has been reported only in some cell lines (Zalcberg et al., 1994).

Amplification of *MDR1* has previously been described in a large number of drug resistant cell lines, with both in situ amplification and amplification in a chromosome other than 7. In a study by Zhou et al. (1996), chromosome painting revealed an hsr derived from chromosome 7 and inserted into 2q in a K562 subline resistant to homoharringtonine (HHT) and demonstrating *MDR1* amplification. The results suggested to the authors that the translocation of *MDR1* to chromosome 2 preceded *MDR1* amplification and that this translocation allowed the *MDR1* gene to escape the normal regulatory control of chromosome 7. It is currently believed that when the *MDR1* amplicon is in a chromosome other than 7, amplification may have begun on chromosome 7, but involved an intermediate step in which the amplified gene resides in a double minute or similar extrachromosomal DNA structure, which reintegrates in a secondary site with the possibility of subsequent additional amplification. In a report on the derivation of double minutes from episomes, which are small extrachromosomal molecular structures of 100–600 KB, Carroll et al. (1988) concluded that the "initial production of episomes, followed by their conversion to DMs and subsequent integration, represent a general molecular chronology for gene amplification." They also proposed that in some cases "gene amplification is a recombination event which deletes a chromosomal region containing a replication origin along with adjacent genes which confer a selective growth advantage." Our results support the involvement of a deletion event and demonstrate that the *MDR1* gene comes under the regulatory control of other genes. There is no evidence, however, of episome or double-minute formation, indicating that amplification does not necessarily proceed from extrachromosome structures to intrachromosomal homogeneously staining regions, as has been suggested by a number of other reports on the relationship between double minutes (dmin) and hsr in vivo (tumors) and in vitro (cell lines) (see Schimke, 1982, for a thorough discussion on gene amplification, dmin, and hsr). Instead, our results suggest that the rearrangement occurs as the first step, and that the rearranged or reassigned *MDR1* gene is then amplified.

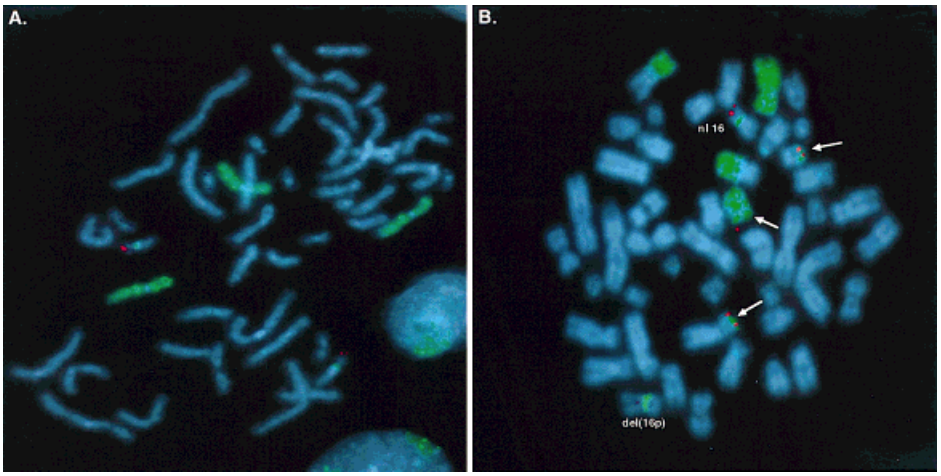

Figure 4. S48–3s and S48–3s/ActD. **A:** WCP7 (green), 16 alpha satellite probe (green), and 16p subtelomere probe in S48–3s parent: no rearrangements. **B:** WCP7 (green), 16 alpha satellite probe (green), and 16p subtelomere probe in S48–3s/ActD: normal 7 × 1, normal 16 × 1, del(16)(pter)x1, t(7;?)x2, t(7;16)x3 (arrows).

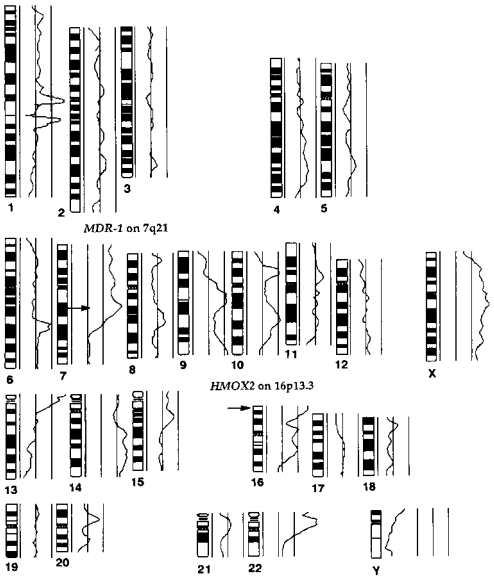

Figure 5. CGH in S48–3s/ActD. Note amplification at 7q21 and 16p13 (arrows).

In summary, the present study describes the cytogenetic findings in drug-resistant cell lines and patient samples. Our results indicate that the initial event in the development of drug resistance is chromosome rearrangement, which brings *MDR1* under the transcriptional control of the promoter from a random, but actively expressed, gene. This causes activation of *MDR1* in cells that normally do not express appreciable levels of *MDR1*. In the present state we observe randomness of the partner of *MDR1* but, if one assumes that the genetic event that results in the juxtaposition of another genomic sequence proximal to *MDR1* is random, then under the selective pressure from chemotherapeutic agents, cells with the best "fitness" (i.e., those with higher drug efflux because of higher levels of *MDR1* expression) are selected independent of the juxtaposed sequences. The ability to detect the initial chromosome rearrangement using FISH with

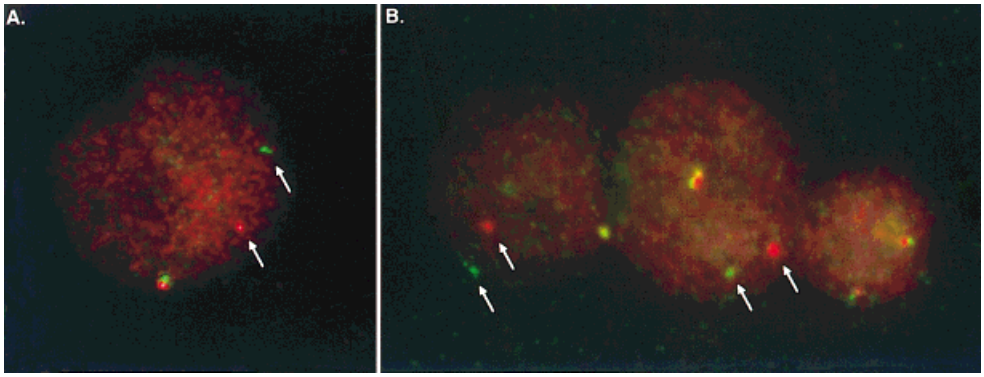

Figure 7. BAC hybridization in ALL patients. Merged signal = no rearrangement; split signal = rearrangement within 7q. Arrows indicate split signals. **A:** ALL patient 1. Split signal indicates rearrangement within 7q. **B:** ALL patient 2. Split signal indicates rearrangement t(1;7).

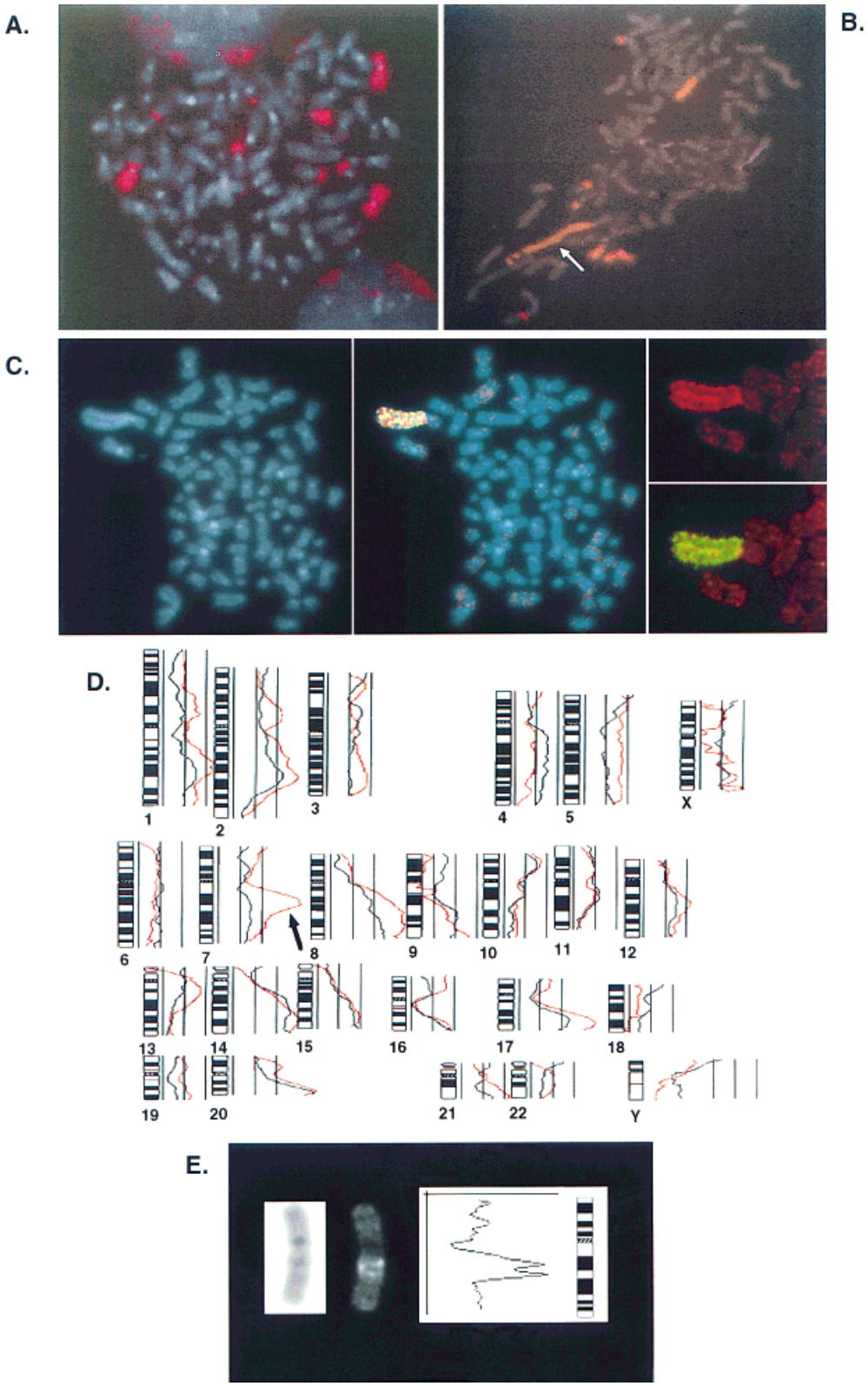

Figure 6. MCF-7 parent and MCF-7 TX400. **A:** WCP7 in MCF-7 parent: multiple rearrangements including nine clonal abnormalities. **B:** WCP7 in MCF-7 TX400: multiple rearrangements as in parent, plus chromosome 7 hsr (arrow). **C:** FISH with BAC-derived probes in MCF-7 TX400. Left panel shows DAPI stain of metaphase exhibiting hsr. Middle panel shows merged *MDR1* (red) and CASP (green) amplification in hsr. Top right panel shows *MDR1* (red) in hsr. Bottom right panel shows CASP (green) amplification in hsr. **D:** CGH in MCF-7 and MCF-7 TX400: superimposed CGH profiles of parent MCF-7 (black) and MCF-7 TX400 (red). Note double peak (in red) of amplification at 7q21 and 7q22 in the MCF-7 TX400 profile (arrow). **E:** CGH of chromosome 7 in MCF-7 TX400 demonstrating amplification of both the region containing *MDR1* at 7q21 (upper peak) and that containing CASP at 7q22 (lower peak). There is reduction or absence of amplification of the intervening sequences.

BAC-derived probes has the potential to be a clinically useful tool for detecting, and possibly predicting, the acquisition of drug resistance in patients.

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